20030128119

MUSCLE COMPOSITION IN INFECTION

Final Report

November 1978

by

Buford L. Nichols
G.L. Bilbrey
C. F. Hazlxwood
S. L. Kimzey
C. T. Liu
W. R. Beisel

MAR 0 4 1987

AD-A177 589

87   3   2   124

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD A177 575	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Muscle Composition in Infection		5. TYPE OF REPORT & PERIOD COVERED Final Report 15 June 1974-15 December 1974
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Buford L. Nichols, M. D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-74-C-4079
9. PERFORMING ORGANIZATION NAME AND ADDRESS Baylor College of Medicine Houston, Texas 77030		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62760A 3A762760A834.01.920
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE November 1978
		13. NUMBER OF PAGES 43
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Same as Block 20

18. SUPPLEMENTARY NOTES

Supplementary Notes - NA

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Electrolyte Balance, Serum Electrolyte Concentration ; Infectious Diseases, Salmonella typhimurium, Sepsis, Serum Sodium and Potassium; Muscle Sodium and Potassium and Water, Renal Excreation of Sodium Potassium and Water, Fever, Water Retention. (cont'd on back)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Alterations occur in human electrolyte balance and serum concentration during infectious diseases. To explore these alterations in greater detail, electrolyte metabolism has been investigated in rhesus monkeys with a sublethal illness induced by intravenous inoculation with Salmonella typhimurium. Response to illness was evaluated by measurements of serum and muscle electrolyte composition and renal function. In the animals with ad libitum dietary intake, a loss in muscle and serum potassium concentrations became (cont'd on reverse side)

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE

19. (Cont'd)

Sodium Retention, Intracellular Increase in Water and Sodium, Convalescence, Weight Loss, Urine Excretion, Asynchrony of Return of Renal Function, Overhydration of Muscle, Intracellular Electrolyte Composition of Muscle, Muscle-Protein-Lipid Concentration.

20. (Cont'd)

evident within 24 hours after inoculation during the febrile phase of illness. Serum and muscle K concentrations returned to normal after five days of illness. Sodium and water content of muscle responded in a more complex pattern. During fever, muscle sodium and water increased and sodium concentrations in serum and urine were elevated. This appeared to be due to the greater relative retention of water than of sodium in muscle which led to increased concentrations of sodium in serum and in the renal filtered load. The intracellular nature of the increased muscle water was documented with direct determination of fiber composition by Electron Probe Micro-analysis. During convalescence, a renal retention of sodium was marked and overlapped the period of weight loss and increasing urine volume. This asynchrony in return of normal renal function appeared to be the cause of relatively large swings in plasma sodium concentrations during the early convalescent period. During this period, muscle water and sodium concentrations were increased when serum sodium concentrations were reduced and reduced when serum sodium was elevated. These investigations indicate that the altered serum concentrations in infectious diseases are a complex algebraic sum of renal and extrarenal factors which control electrolyte metabolism, and further, that some of the most remarkable alterations occur during early convalescence as renal function returns to normal.
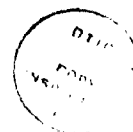
ELECTROLYTE METABOLISM IN RHESUS MONKEYS WITH EXPERIMENTAL SALMONELLA SEPSIS

B. L. Nichols[1], G. L. Bilbrey[2], C. F. Hazlewood[1], S. L. Kimzey[3],

C. T. Liu[2], and W. R. Beisel[2]

1. Section of Nutrition and Gastroenterology
   Department of Pediatrics
   Baylor College of Medicine
   Houston, Texas

2. U. S. Army Medical Research Institute
   of Infectious Diseases
   Ft. Detrick, Maryland

3. Cellular Analytical Laboratory
   L. B. Johnson Space Center
   National Aeronautics and Space Administration
   Houston, Texas

ABSTRACT

Alterations occur in human electrolyte balance and serum concentration during infectious diseases. To explore these alterations in greater detail, electrolyte metabolism has been investigated in rhesus monkeys with a sub-lethal illness induced by intravenous inoculation with Salmonella typhimurium. Response to illness was evaluated by measurements of serum and muscle electrolyte composition and renal function. In the animals with ad libitum dietary intake, a loss in muscle and serum potassium concentrations became evident within 24 hours after inoculation during the febrile phase of illness. Serum and muscle K concentrations returned to normal after five days of illness. Sodium and water content of muscle responded in a more complex pattern. During fever, muscle sodium and water increased and sodium concentrations in serum and urine were elevated. This appeared to be due to the greater relative retention of water than of sodium in muscle which led to increased concentrations of sodium in serum and in the renal filtered load. The intracellular nature of the increased muscle water was documented with direct determination of fiber composition by Electron Probe Microanalysis. During convalescence, a renal retention of sodium was marked and overlapped the period of weight loss and increasing urine volume. This asynchrony in return of normal renal function appeared to be the cause of relatively large swings in plasma sodium concentrations during the early convalescent period. During this period, muscle water and sodium concentrations were increased when serum sodium concentrations were reduced and reduced when serum sodium was elevated. These investigations indicate that the altered serum concentrations in infectious diseases are a complex algebraic sum of renal and extrarenal factors which control electrolyte metabolism, and further, that some of the most remarkable alterations occur during early convalescence as renal function returns to normal.

INTRODUCTION

In prospective studies of infections in human volunteers and monkeys, a series of metabolic responses have been observed which exaggerate the effect of anorexia on electrolyte and water balance. The earliest renal response in monkeys was a depressed free water clearance evident within two hours after intravenous inoculation with virulent pneumococci (1). In the human, this was followed by a two or three day period of renal wastage of sodium and chloride without change in body weight (2). A two or three day period of sodium retention associated with maximal aldosterone secretion then occurred which coincided with the onset of clinical recovery, rapid diuresis and weight loss (3,4,5).

Reduced concentrations of serum Na and Cl during infections have been reported in a number of clinical studies (5). In prospective human studies of tularemia and Q fever (2), falls in serum Na and Cl concentrations were observed in the first days following recovery from the febrile response. These were transient but as profound as 10 mEq/L. In tularemia, hyponatremia and hypochloridemia occurred simultaneously with maximum urinary aldosterone excretion (2). Urinary potassium concentrations increased and K balance became negative with the onset of the febrile response in experimental infections (2). Urine K losses were poorly related to urinary Na concentrations. In the experimental human infections with tularemia, sand fly fever and Q fever, no significant alterations in serum K concentration were observed.

Muscle was observed to participate in the altered electrolyte metabolism of several children who developed incidental infections while undergoing metabolic investigations during nutritional recovery (6). Because muscle plays a major role in extrarenal electrolyte metabolism, a series of experiments were designed to explore the role of skeletal muscle in the altered serum and urinary electrolyte concentrations discussed above. A descriptive investigation of muscle and serum chemical composition in rhesus monkeys infected with Salmonella typhimurium was conducted. These observations were extended in additional monkeys to explore physiological

correlates at the integrated and cellular levels.

MATERIALS AND METHODS

Animal Model

Nineteen rhesus monkeys (Mucaca mulatta) with body weights between 2.5 and 4.5 kg were maintained on Purina monkey chow during a six month isolation period prior to experimentation. Two groups resided in individual cages throughout the study. An additional group was restrained in metabolic chairs. During the experiment, the animals received their usual water and diet ad libitum. Before all manipulative procedures, the monkeys received an intramuscular injection of 1 mg/kg of a tranquilizer, phencyclidine (Sernyl[R]).

Test Procedure

Stock culture of Salmonella typhimurium was stored frozen (-60°F) in brain-heart infusion broth containing 17% glycerine by volume. Prior to inoculation, a sample of stock culture was thawed at room temperature. Nutrient agar slants were inoculated with 0.2 ml of stock culture and incubated at 37°C for 18 hours. The nutrient agar slant was washed with phosphate buffered saline plus 1% normal rabbit serum and gently agitated. The yield of organisms per ml of wash was titrated reproducibly by varying the amount of saline and serum added to the agar slant. Three ml yielded approximately 1×10^8 organisms per ml. One ml of this titered inoculum, when administered intravenously to the rhesus monkey, consistently produced a febrile, non-lethal illness. Blood cultures were obtained at the time of each venapuncture during the experiments to document the presence of bacteremia.

Experimental Procedure

Samples of skeletal muscle weighing 5 - 15 mg were obtained with a Baylor muscle biopsy needle (Popper and Sons, Inc., New York, N.Y.) from the anterolateral thigh of the monkey. The exact time of biopsy was recorded with a stop watch. Serial weights of muscle specimens were recorded immediately after biopsy by using a Cahn Electrobalance (Cahn Inst. Co., Paramont, Ca.) in order to calculate the initial wet weight of the biopsy. The samples were then analyzed for Na, K, Cl, H_2O, myofibrillar

nitrogen, collagen nitrogen and total lipids by methods previously described (7). Serum ion concentrations were measured by the same techniques. Serum osmolality was measured by freezing point depression (Advanced Instruments, Inc., Newton Highlands, Mass.).

Muscle samples from Group III animals were also analyzed by electron probe micro-analysis (8). Using fine scissors, 1 mm blocks of muscle were removed from the biopsy sample. These were frozen in liquid propane within 60 seconds of the initial exposure of the sample to ambient conditions. The frozen samples were then transferred to a cryostat where they were sectioned at 8μ thickness, picked up on polished silicone discs and lyophilized. Gelatine standards were prepared in the same manner.

The electron probe utilized was an Applied Research Laboratories EMX-SM located in the Cellular Analytical Laboratory of the L. B. Johnson Space Center. Three crystal wavelength dispersive spectrometers were used for the simultaneous assay of potassium, sodium and chloride. Silicone substrate signal was monitored with an additional non-dispersive spectrometer in order to determine the tissue density. Individual fibers were scanned using an electron beam with a measured diameter of 1.5μ, a 10 KV accelerating potential, and a sample emission current of $0.1\mu A$. Calibration curves were linear for potassium, sodium, chloride and tissue density.

Experimental Protocols

Three experiments are reported. In the first, Group I, five individually caged monkeys received intravenous inoculations of 10^8 S. typhimurium organisms. These monkeys developed a febrile illness with a subsequent 10% loss in body weight. Daily rectal temperatures (Brown Instruments, Philadelphia, Pa., Model Y153 x 60) and body weights were recorded (Shadowgraph Scale, The Exact Weight Scale Co., Columbus, Ohio). Blood samples were cultured and muscle samples obtained for analyses at 6, 24, 48, 72, 120, 168, 240 and 336 hours after inoculation. With the exception of the 6 hour observation, all sampling was done between 9:00 and 11:00 A.M. In order to determine the normal range of muscle composition, these same monkeys had control samples of muscle obtained at 72, 48, 24 and zero hours before the inoculation. The samples at -48,

-24 and zero hours are referred to as <u>experimental controls</u>. One additional monkey was subjected to the same experimental protocol as the Group I animals. This monkey received an intravenous injection of 1 ml of sterile saline. Results from this monkey have been included among the experimental controls.

From the viewpoint of the results of the Group I investigation, questions arise concerning the response of homeostatic controls to <u>S. typhimurium</u> septicemia. To explore these, an investigation was conducted in five additional monkeys, <u>Group II</u>, restrained in metabolic chairs and studied in the conscious state. Additional animals were investigated with this second protocol, however, only those monkeys with a pure blood culture of <u>S. typhimurium</u> were accepted for inclusion in this analysis. The chronic ureteral cannulations required for this experiment resulted in asymptomatic bacteremia with other organisms in all six saline inoculated control animals and mixed infections in three of eight monkeys inoculated with <u>S. typhimurium</u>.

Surgical procedures were accomplished 72 hours prior to bacterial inoculation (0 hours). Anesthesia was induced with sodium thioamytal given intravenously. Using conventional sterile techniques, a midline abdominal incision was performed. Poly-ethylene catheters (PE-160) were inserted into both ureters and positioned in each renal pelvis. The external catheter tips were exteriorized through separate abdominal sites and attached to a sterile, closed system for urine collection. Through a separate groin incision, a venous catheter (PE-100) was positioned high in the inferior vena cava via the femoral vein for infusions required for physiologic function tests. In addition, a teflon catheter (I.D. 0.042 in.) was inserted via the femoral artery into the abdominal aorta for measurement of blood pressure and blood sampling. Mean arterial blood pressure (MABP) was measured with Statham pressure transducer (Statham Instruments, Inc., Oxnard, Calif. Model P23D6) connected to a Brush recorder (Gould, Inc., Cleveland, Ohio). The arterial and venous catheters were flushed twice daily with 5 ml of sterile saline solution containing 50 u/L of heparin. A thermocouple was placed deep in the paraspinal muscles at the level of the first lumbar vertebra for continuing monitoring of body temperature (Brown Instruments, Philadelphia, Pa.,

Model Y153 x 60).

Following surgery (-72 hours), the monkeys were studied at -48, -24, 0, +72 and +120 hours after inoculation with 10^8 S. typhimurium organisms at time zero. In addition, blood was withdrawn daily for determination of serum electrolytes, osmolality, and creatinine. Daily collections were made for determination of 24 hour urine volume, osmolar and electrolyte excretion and creatinine excretion. Blood and urine cultures were obtained at 48 and 96 hours.

Because of the blood loss required by these physiologic determinations, daily arterial hematocrits were determined. The Group II monkeys were transfused with pooled monkey red blood cells at -24, +48 and + 96 hours after inoculation in order to maintain an hematocrit above 25%.

Plasma and extracellular volume determinations, cardiac output, renal function and muscle sampling were accomplished at 0, 72 and 120 hours after inoculation. Plasma volume was determined from optical density of serum 10 minutes after intravenous injection of 4 mg/kg of Evans blue dye. Extracellular volume was determined by the method of Walser using $Na_2S^{35}O_4$ (Amersham-Searle). Twenty-five ₁c was injected I.V. and plasma samples obtained at 0, 15, 20 and 25 minute intervals. Counting was done in a Liquid Scintillation System (Nuclear Chicago, 720 series) and results extrapolated to time 0 of determination of equilibrium distribution. Cardiac output was determined by indicator-dilution curves following rapid injection of 5 mg of cardiogreen dye (Hynson, Wescott and Dunning, Inc., Baltimore, Md.) using the Gilford 103 IR densitometer, 104 dye curve computer and 105 constant flow pump (Gilford Instruments, Oberlin, Ohio). Output was calculated as ml/min by the formula:

$$\text{Cardiac output} = \frac{I}{K} \frac{(60)}{(AF)}$$

where I was 5 mg of dye injected, AF the area factor from the computer, and K was a constant derived from known concentrations of the dye in the pooled monkey blood. Renal function was tested after appropriate loading doses of a solution of inulin and PAH (1). A subsequent constant infusion of 0.125 ml/min maintained blood concentrations in the range of 10 - 20 and 1 - 2 mg/DL plasma respectively. After a 90

minute equilibration period, four hourly clearances were determined. Calculations were standard clearance formulae. Inulin and PAH were measured in a Technicon Autoanalyzer (Technicon Corp., Rochester, N.Y.) using a modification of the technique of Dearborn and Harvey (1). Creatinine analysis employed a modification of the procedure of Folin and Wu (1).

When the data from the initial two experiments were compiled, it was observed that alterations in electrolyte composition of serum and muscle at 120 hours after inoculation presented an unusual pattern. This was reinvestigated in a third experiment. Eight additional individually caged monkeys, Group III, received intravenous inoculations. Four received 5×10^8 and four 1×10^8 S. typhimurium organisms. The experimental procedures were carried out as Group I at 0 and 120 hours after inoculation. Several additional analyses of serum concentrations were added to this third experiment. They include hematocrit, serum protein, BUN, Ca, Mg, P and blood pH.

Because of the effect of the experimental procedures observed in the collection of the serial samples in the experimental controls, the first samples (72 hours before inoculation) from Group I and the time 0 samples for Group II are pooled as the absolute controls.

Statistical Procedures

Mean values are reported with ± one standard deviation. All data groups were tested by the t test for significance of differences. When the p value is less than 0.05, mean differences are stated to be significant. Relationships between observations were tested by regression analyses or the Chi Square test.

RESULTS

The intravenous inoculation of rhesus monkeys with 10^8 S. typhimurium organisms resulted in a febrile illness without gastrointestinal disturbance. The mean body temperature and body weight of the Group I animals are charted in Figure 1. Mean body weights in the Group II monkeys are illustrated in Figure 2. Body weights for Group III are found in Table II. Blood cultures became positive for S. typhimurium

in all inoculated monkeys*

In the Group I animals, a series of changes were observed in serum electrolyte concentrations (Figures 3, 4 and 5). Serum sodium and chloride concentrations followed similar patterns after inoculation of the monkeys. Both ions were significantly elevated within six hours after the zero observation; an elevation of approximately 10 mEq/L persisted through the first 72 hours of infection. Between 72 and 120 hours, both fell to values not statistically different from the baseline. A significant rise was again observed at 168 hours in sodium and chloride concentrations but returned to control values by 240 hours. The relationship to serum sodium in the Group I experiment is reflected in a regression equation: Serum Cl mEq/L = Serum Na ⋅ 0.73 - 3 (r = 0.90, n = 46).

Serum potassium concentrations were significantly lowered from the zero hour observations at six through 120 hours. Between 120 and 168 hours after inoculation, there was a significant rise of 1 mEq/L. Thereafter, levels were not significantly different from those before the study began. Muscle potassium concentration in mEq/Kg fat free dry weight (FFDW) fell during the initial hours after inoculation reaching statistical significance at 72 hours. Between 120 and 168 hours after the beginning of the experiment, mean muscle K concentration increased to control values which persisted through the remainder of the experiment. A parallelism existed between the serum and muscle K concentrations. This is reflected in Figure 4 which

---

*Although similar in most respects, there were minor differences between the Group I and III experiments. The Group III animals weighed 0.5 kg more at the start of the experiment and had a 10% loss of initial weight by 120 hours, a significantly greater loss of weight than was observed at this interval in Group I monkeys. All of the Group III animals had persistent bacteremia at 120 hours, whereas only two of the five Group I monkeys had S. typhimurium cultured from the blood at this time. These data suggest that the infection may have been more severe in the Group III experiments.

illustrates this relationship for Group I. Regression analysis revealed that muscle K mEq/Kg FFDW = Serum K ' 36.6 + 216 (r = 0.59, n = 40). The Group III data also follow this relationship, without altering the equation (Figure 6).

In Group I, muscle sodium concentrations (Figure 3) did not reflect serum concentrations. Six hours after inoculation, there was a significant fall in muscle Na at a time when serum concentrations rose significantly. There was an increase in variation among the sodium concentrations in muscle between 24 and 120 hours after inoculation. This occurred because half of the animals developed muscle sodium levels approximately twice those of the experimental controls and time zero observations. Between 120 and 168 hours of illness, muscle sodium decreased from the highest to the lowest levels observed during the Group I experiment. This fall of approximately 200 mEq/Kg FFDW was statistically significant. It was associated with a significant rise of 26 mEq/L in serum sodium concentration. Additional reciprocal changes in serum and muscle Na were observed at 240 and 336 hours post inoculation. The inverse relationship between muscle and serum sodium concentrations between 120 and 336 hours after inoculation is reflected in a regression equation: Muscle Na mEq/Kg FFDW = 371 - serum Na mEq/L ' 0.86 (r = 0.49, n = 20). This relationship did not exist between serum and muscle sodium concentrations between zero and 120 hours.

Muscle chloride concentrations (Figure 5) followed those of Na. The changes were not as large as those noted in sodium concentration, however, the reciprocal relationship between muscle and serum Cl concentrations was present between 120 and 336 hours after inoculation; muscle Cl mEq/Kg FFDW = 392 - serum Cl mEq/L·2.73 (r = 0.4, n = 20).

The effect of sepsis on myofibrillar nitrogen, collagen nitrogen and neutral lipid is illustrated in Figure 7. During the early stages of infection, there was a transient drop in myofibrillar nitrogen, significant at 6 and 24 hours. At the same time, a relative increase in collagen nitrogen was observed. Tissue lipids increased significantly during the first 72 hours after inoculation, then fell to control levels at 120 hours.

Water content of muscle also changed during the period after inoculation. There was an initial fall in muscle water followed by a mean rise of significant proportions at 72 and 120 hours. The water content fluctuated between 120 hours and 336 hours with the same pattern as observed for muscle sodium. There was a relationship between muscle water and sodium; muscle Na mEq/Kg FFDW = 3.1 + muscle water 1/Kg FFDS· 0.0037 (r = 0.62, N = 40). If only the observations between 120 and 336 hours were used, the regression coefficient increased to 0.71.

Group II monkeys were subjected to surgery, chronic cannulation and chair restraint. They also received intravenous fluid infusions of 5.5 hours duration on the days when renal functions were measured. The course of infection in this group was identical to that of the Group I monkeys. Instead of weight loss, these restrained animals gained an average of 387 gms and became visibly edematous (Figure 2). The effect of this experimental protocol on muscle K, Na and H_2O was indistinguishable from that observed in the Group I animals at 120 hours after inoculation* Muscle chloride in the Group II monkeys was significantly higher at 120 hours; 232 ± 90 vs 132 ± 38 mEq/kg FFDW in Group I. Serum K was already reduced at -48 hours in the operated animals and remained low except for transient increases at 24 and 144 hours. Serum Na fell in Group II, in distinction to the early rise observed in the Group I monkeys who did not receive water loads. Serum chloride concentrations demonstrated an identical rise in both groups after inoculation, however, this lacked statistical significance in Group II because of variations in serum concentrations at 0 hours.

As illustrated in Figure 8, after infection was induced, the three day mean urine volume increased significantly from 123 ± 62 to 237 ± 89 ml/24 hours. There was a transient decline in urine volume during the third day. Volume thereafter increased progressively until 144 hours of observation. During the first 48 hours, the urinary excretion of sodium appeared to parallel urinary volume. At 72 hours, there was a fall in urine Na concentration which lasted through the fifth day of infection (120 hours). During the first 48 hours, sodium excretion was significantly increased

---

*Figures illustrating these changes in muscle and serum in the Group II monkeys are available from the authors or from the Houston Academy of Medicine Library, Texas Medical Center, Houston, Texas 77030.

by an average of 5 mEq/day when compared with the baseline and 72 to 120 hour collections. Urinary potassium excretion was significantly elevated by 24 hours, an increase in excretion averaging 5 mEq/day during the period following inoculation. The K loss seemed unrelated to urine volume or sodium excretion.

When examined on a daily basis (Figure 9), glomerular filtration rate as reflected in creatinine clearance persisted at basal levels until the third day (72 hour collection). At this time, there was a 30% reduction in GFR . This reduction persisted through the fifth day (120 hours). The reduction in GFR coincided with sodium conservation (Figure 8). Antidiuresis, as measured by daily total C_{H_2O}, also increased between 72 and 120 hours. Despite the increasing urine volume during the week after inoculation, antidiuresis was present except for a relatively isosmotic urine excreted during the 24 hours following inoculation of the virulent organisms. Serum osmolality was reduced by 16 mOsm/Liter from baseline observations during the entire seven days after inoculation.

Explanations for the alterations in renal function and muscle composition were not found in the physiologic investigations (Table I). No statistical differences were found in the extracellular space, or plasma volume. No changes in cardiac function could be documented. Inulin clearance measurement of GFR confirmed the creatinine clearance observations indicating a 30% reduction in glomerular filtration at 72 and 120 hours after inoculation. The alterations in inulin clearance, however, did not achieve statistical significance. No significant alteration could be documented in effective renal plasma flow (PAH clearance) or filtration fraction.

To explore the intracellular basis for the above described muscle changes more fully, eight additional caged monkeys (Group III) were studied at 0 and 120 hours after inoculation. The range of determinations were expanded in blood and muscle (Table II). The animals all became febrile with the infection. At 120 hours after intravenous inoculation, body weight had fallen to 90 ± 3% of that at the start of the experiment. Among the analyses performed on blood, the following demonstrated significant reductions: Sodium, 7 mEq/L, potassium, 0.9

mEq/L; chloride, 7 mEq/l; calcium, 0.6/DL and phosphorus 1.6 mEq/DL. The hemato-crit fell by 6 vol %. Serum proteins, blood urea nitrogen, osmolality, pH and magnesium concentrations were unchanged from zero hour observations.

Whole muscle analyses revealed a significant decrease in K, 45 mEq/kg FFDW. Potassium concentration on a whole muscle basis fell from 95 to 86 mEq/kg FFWW, however, this did not achieve statistical significance. When assayed on an intra-cellular basis, K fell from 104 to 85 mEq/kg WW of fiber. This was a significant change. In addition, intrafiber water content increased from 2.3 to 3.6 mEq/kg DW of fiber.

There have been few studies of muscle electrolyte metabolism in the monkey. It is of value to determine the effects of the experimental procedures, other than the test inoculation of S. typhimurium, on these animals (Table III). The absolute controls (Group I, -72 hours and Group III, 0 hours), with no prior intervention, were compared with the experimental controls (Group I, -48, -24 and 0 hours) who had been subjected to three days of testing and the operated controls (Group II, -48, -24 and 0 hours) (Table III). Small but significant changes occurred in serum Na and Cl concentrations in response to experimental procedures. These changes are reflected in alterations in muscle H_2O, Na and Cl. Only serum and muscle K concen-trations were not influenced by the experimental procedures (Group I), however, serum K was reduced in the operated controls (Group II). Because of these small, but nonetheless significant, biological responses to experimental procedures which consisted of sedation, blood and muscle sampling and temperature and weight measure-ments, the time zero sample, the first of the experimental observations, is used as the basis for comparison in Groups I, II and III.

DISCUSSION

The intravenous inoculation of the rhesus monkey with 10^8 Salmonella typhimurium produced a uniform sub-lethal, self-limited febrile illness. The relationship be-tween inoculation and illness was confirmed by the persistence of S. typhimurium in blood cultures. All of the Group I animals had septicemia persisting to 72 hours;

the Group II to 96 hours; and Group III had persistence to 120 hours following inoculation.

After 72 hours of infection, the average body weight began to fall in the Group I animals, reaching levels of significance at 168 hours and resulting in a total loss of 10% of body mass at 336 hours post inoculation. In the Group III animals, the loss of body mass was more rapid, reaching 10% of control weight at 120 hours. The chair-restrained Group II animals gained weight because of the water loading and developed visible edema.

Six hours after inoculation of the Group I monkeys with S. typhimurium, serum concentrations of Na and Cl increased by approximately 10 mEq/L. At the same time, serum K fell by 0.8 mEq/L and there was a transient loss in muscle water of 450 ml/Kg of dry muscle solids. This was associated with a significant fall in myofibrillar nitrogen and increase in muscle collagen nitrogen and lipid. At this time, muscle Na and Cl/Kg FFDW fell significantly and K concentrations were relatively unchanged. There was no evidence of total body dehydration as reflected in acute loss of body mass at this period.

The second phase of infection was associated with the febrile response which lasted from 24 hours to 72 hours after inoculation. The trend in serum electrolyte concentrations, already evident at six hours (increase in Na and Cl and fall in K) was persistent. The alterations in muscle, however, were very different. During this period, muscle water and sodium increased above the baseline, muscle chloride concentrations returned to the original levels but muscle K concentrations continued to decrease, becoming statistically different from the zero hour observations at 72 hours post-inoculation. Muscle myofibrillar and collagen nitrogen returned to original concentration in the dry muscle solids by 72 hours but muscle lipid continued to increase up to that point. The water loading of the Group II monkeys prevented the elevation of serum Na which had been observed in Group I. In Group II, the postoperative serum K concentrations, which were low at 0 hours, remained low throughout the study. During infection, muscle compo-

sition was very similar to that observed in the Group I animals, indicating that although the water loading in Group II altered serum concentrations, it did not alter the muscle response. This suggests that muscle hydration was independent of extracellular sodium or total osmolar concentration.

In convalescence, after lysis of fever, muscle sodium and chloride concentrations which had fallen significantly in Group I during the febrile period, rose to very high levels at 168 hours. Muscle water, sodium and chloride concentrations all fell significantly during the same interval. The increase in serum Na and Cl must have been due, in part, to the delivery of these tissue components into the extracellular fluids. This shift was associated with a diuresis as evidenced by an average weight loss of 9%. Urine volume increased transiently in Group II monkeys at 24 hours but sustained diuresis did not begin until the fourth day after inoculation. Saluresis was evident at 24 and 48 hours but was followed thereafter by sodium retention. The rise in serum Na and Cl concentrations in Group I must reflect the relative retention of these ions during early diuresis of fluids delivered from the tissues after 72 hours of illness. A fluctuating inverse relationship observed between serum and muscle Na and Cl persisted throughout the remainder of the Group I convalescent period. The serum concentrations fell at 240 hours when muscle concentrations of Na, Cl and H_2O again increased. Fluctuations in serum sodium concentration have been observed during the convalescent period in other experimental infections such as Q fever (2). The periodicity of the reciprocal shifting of ions and water out of muscle observed in the present study cannot be determined because of the long sampling intervals during convalescence. It is not certain that recovery was complete at 336 hours (14 days) following inoculation; serum Na and Cl levels continued to be significantly greater than those at time zero, muscle Na and Cl were significantly less. Muscle and serum K were fully recovered after 168 hours. Muscle water, myofibrillar nitrogen and neutral lipid concentrations were equivalent to the original observations but muscle collagen nitrogen was low at the time of the final observation.

The response of muscle K to <u>S. typhimurium</u> septicemia appears to be related to renal wasting of this ion during the illness. It is clear that serum K concentrations are an important determinant of muscle K concentration, a linear correlation exists between the two in experimental K deficiency in rats. The relationship is similar in this study (Figure 6). It is likely that renal K wasting·is produced by the hypermineralocorticoidism known to occur in the febrile period of infectious diseases. Potassium deficiency could explain the increase in muscle sodium observed in the febrile phase of the illness, however, it does not explain the increase in muscle water. Overhydration of muscle is not found in experimental K deficiency. Eight animals in the Group III received a similar inoculation of <u>S. typhimurium</u> organisms and were studied at zero and 120 hours of illness. Muscle responses in Group III were similar to those observed in the Group I monkeys. Muscle potassium was reduced by 50 and 45 mEq/kg FFDW in the Group I and III monkeys respectively. Muscle water increased 0.4 and 0.3 liters/kg FFDW in these two groups. The cellular nature of these alterations was investigated by use of the Electron Microprobe. These observations (Table II) indicated that fiber hydration was significantly increased at 120 hours after infection. Intrafiber potassium correlated with fiber hydration (Figure 10): K, mEq/kg whole fiber = 126 - H_2O, L/kg dry fiber hydration. Muscle K/kg FFDW correlated with serum K, both in Group I and II animals, offering evidence that a portion of the reduction in muscle K was due to K deficiency which was reflected in both serum and tissue. Potassium deficiency, however, is associated with constant muscle hydration and a replacement of intracellular K with Na on an approximately equimolar basis. In the Group III experiment, muscle fiber hydration was increased and intracellular potassium was not replaced by sodium. From the intracellular relationship revealed in Figure 10, it is apparent that intracellular K was diluted by a solution containing approximately 20 mEq of sodium and 10 mEq of chloride per liter. Although the reduction in muscle K from potassium deficiency must play a role in the loss of cellular content of this ion, intracellular K is better correlated with intra-

cellular water (r = 0.74) than extracellular potassium concentration (r = 0.41). Therefore, 120 hours after inoculation, the overhydration of the muscle fiber plays a more dominant role in the reduction of muscle K than does K deficiency. The role of extracellular osmolality on the observed increase in fiber hydration seems negligible; there was no decrease in serum osmolality at 120 hours of infection in the Group III monkeys. By exclusion, the intracellular hydration change in muscle must be a consequence of altered cellular physiologic factors. One of these is revealed by calculating the Na and K fiber content on a dry weight basis. The sum of Na + K/kg dry fiber increases from 400 $\pm$ 71 to 475 $\pm$ 56. When compared with fiber water, the regression coefficient for this relationship is 0.83.

As a background to the study of muscle electrolyte composition several organic components of muscle were analyzed. Total muscle lipid increased four-fold during the 72 hours after inoculation then returned to baseline levels. In other studies, the alterations in lipid metabolism of the monkey with S. typhimurium sepsis have been found to include a five-fold rise in serum triglycerides (48 hours) and an inhibition of triglyceride clearance from blood (9,10). Although muscle lipids have not been previously reported in this illness, it is evident that muscle accumulates lipids during this period of altered triglyceride metabolism.

Muscle myofibrillar nitrogen was reduced by approximately 20% 24 hours after inoculation. There was a compensatory rise of collagen nitrogen in the fat free dry muscle solids. After 48 hours of infection, both nitrogen fractions could not be differentiated from baseline observations. Studies in rats infected with S. typhimurium document a flux of amino acid from muscle to liver (11). Some are incorporated into acute phase serum proteins, however, the major portions appear to enter energy producing pathways (11,12). This flux of amino acids from muscle to liver, which peaks at 24 hours, is associated with marked muscle protein catabolism and increased anabolism in liver and kidney (5).

CONCLUSIONS

Potassium deficiency, reflected in serum and muscle K concentrations, was

present during the febrile period of experimental septicemia in monkeys. Over-
hydration of muscle was evident within 24 hours after inoculation with S. typhi-
murium. This increased in association with an accumulation of muscle Na during
the febrile period. During convalescence, there were reciprocal swings of serum
and muscle sodium, chloride and water content which presumably reflect the stepwise
return of extrarenal and renal mechanisms to normal.

The overhydrated state of muscle in the febrile and convalescent periods is in-
consistent with the known responses of experimental animals to K depletion. An
electron microprobe evaluation of intrafiber concentration confirms that intra-
cellular overhydration was present, independent of K deficiency, and that intra-
fiber hydration played the dominant role in altered whole muscle and fiber K con-
centrations. The fact that serum osmolality was unchanged indicates that intrinsic
cellular factors were responsible for the accumulation of muscle water. Within the
muscle fiber an accumulation of a solution of intracellular fluid containing ap-
proximately 20 mEq of Na and 10 mEq of Cl was documented.

During convalescence, a reciprocal divergence between the muscle and serum
sodium concentrations was observed. After 72 hours of illness, muscle Na continued
to increase while serum Na fell. This occurred during the period of maximal sodium
conservation by the kidney. This also coincided with the period of diuresis and
rapid weight loss. The simple explanation of the divergence between serum and mus-
cle Na concentrations may be the shifting of sodium into muscle. At 168 hours,
this shift was reversed with a profound fall in muscle Na and a rise of serum Na
to very high values. Urinary Na excretion became elevated at this period after
inoculation with S. typhimurium. It would appear that urinary excretion paralleled
the oscillation of serum Na concentration as the ion shifted transiently into and
out of muscle. The rise in serum Na at 168 hours was associated with a return of
muscle hydration to normal. The sequence of renal events reflected an interplay
between altered filtered load, due to tissue shifts, and altered tubular function,
in response to aldosterone and antidiuretic hormones, resulting in an overlapping

suite of responses.

Figure 1, Group I: Effect of <u>S typhimurium</u> sepsis on body temperature (F^o) and body weight (% of initial). The six hour observation is indicated independently; all others were made between 9 and 11 A.M. Means and SD are given for each observation.

Figure 2, Group II: Body weight (% of initial) in water loaded, restrained monkeys receiving inoculation at zero hours.

Figure 3, Group I: Effect of sepsis on muscle Na (mEq/Kg FFDW) and serum Na (mEq/L) The mean values different from time 0 are indicated with (*), those different from 120 hours with (÷). All differences are $p < 0.05$ or less.

Figure 4, Group I: Effect of sepsis on muscle K (mEq/Kg FFDW) and serum Na (mEq/L). Symbols as in Figure 3.

Figure 5, Group I: Effect of sepsis on muscle Cl (mEq/Kg FFDW and serum CL (mEq/L). Symbols as in Figure 3.

Figure 6, Groups I and III: Relationship between muscle potassium (mEq/Kg FFDW) and serum potassium (mEq/L). Group I animals indicated in solid circles, Group III in open circles. The calculated slope is muscle K, - serum K ' 36.6 + 216 (r = 0.59, n = 40).

Figure 7, Group I: Effect of sepsis on muscle water, nitrogen fractions and total lipid. Muscle water (L/Kg FFDW) was related to muscle sodium, muscle Na = 3.1 + muscle water ' 0.0037 (r = 0.62, n = 40). Myofibrillar N (MFN) was decreased at 24 hours and collagen N increased. Muscle lipid increased, peaking at 72 hours. Symbols as in Figure 2.

Figure 8, Group II: Effect of sepsis on urinary excretion. Control observations began 24 hours after the animals had surgical implantation of catheters and were restrained in metabolic chairs. The animals received <u>S. typhimurium</u> intravenously at time zero. The urine volume is given in ml/24 hours and Na and K in mEq/24 hours. Means and SD are given for each observation.

volume is given in ml/24 hours and Na and K in mEq/24 hours.
Means and SD are given for each observation.

Figure 9, Group II: Effect of sepsis on renal function. Glomerular filtration
was estimated by creatinine clearance, ml/min. Free water
clearance is a measure of solute-free water removed during
passage through the tubule, µl/min. A negative value indicates
antidiuresis.

Figure 10, Group III: Relationship between fiber water and electrolyte concentrations
as determined by Electron Probe analyses. Potassium: closed
circles, sodium: open circles and chloride: pluses, mEq/kg of
whole fiber. Intrafiber water on abscission, L/kg DS. The
equation used to determine the slopes illustrated were as
follows: Fiber K, mEq/kg whole fiber = 126 - H_2O, L/kg DS · 10.9
(r = 0.74, n = 16); fiber Na, mEq/kg whole fiber = 23 - H_2O
L/kg DS · 1.1 (r = 0.16, n = 16); and fiber Cl, mEq/kg whole
fiber = 11 + H_2O, L/kg DS · 0.1 (r = 0.01, n = 16).

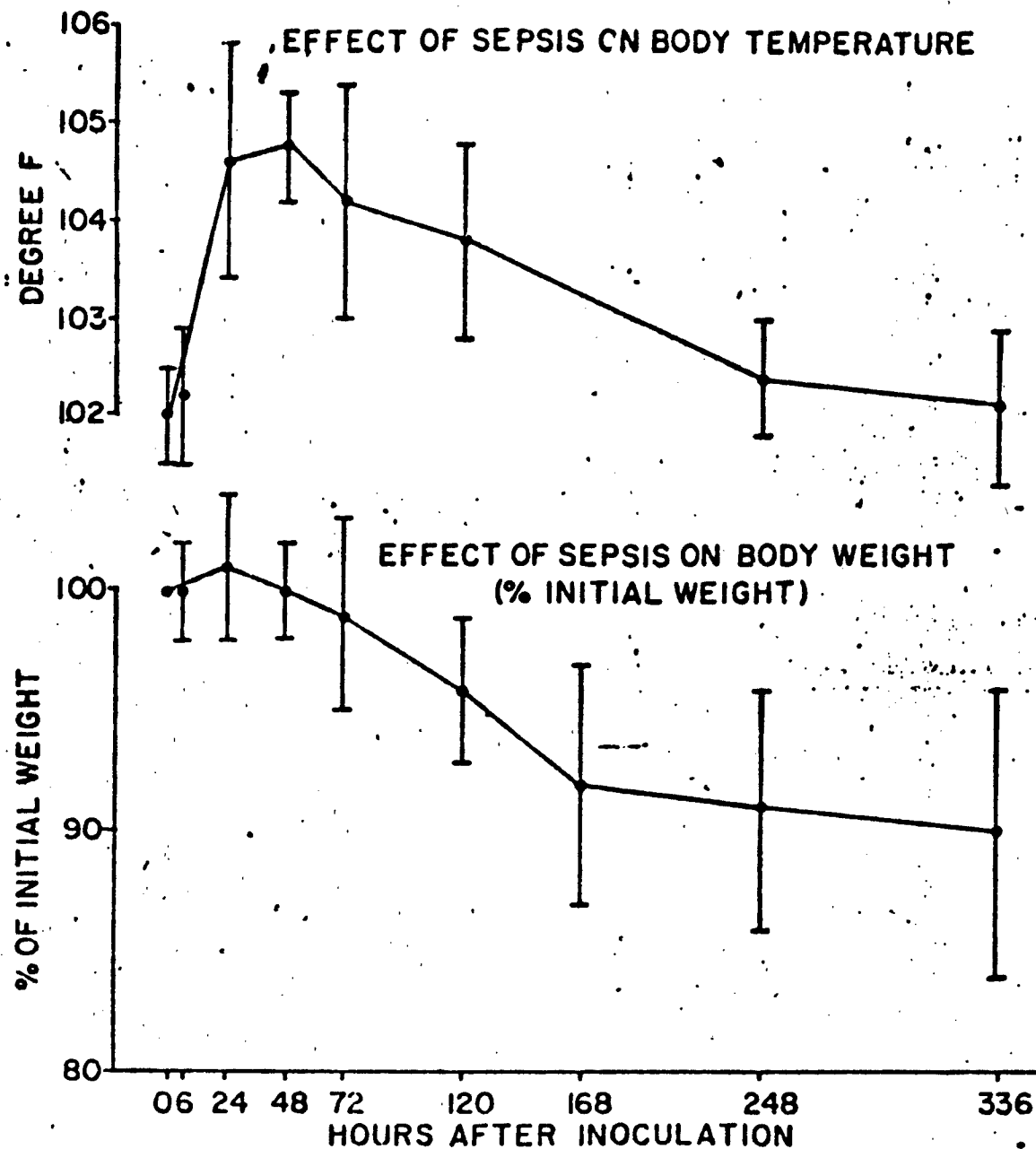EFFECT OF SEPSIS CN BODY TEMPERATURE
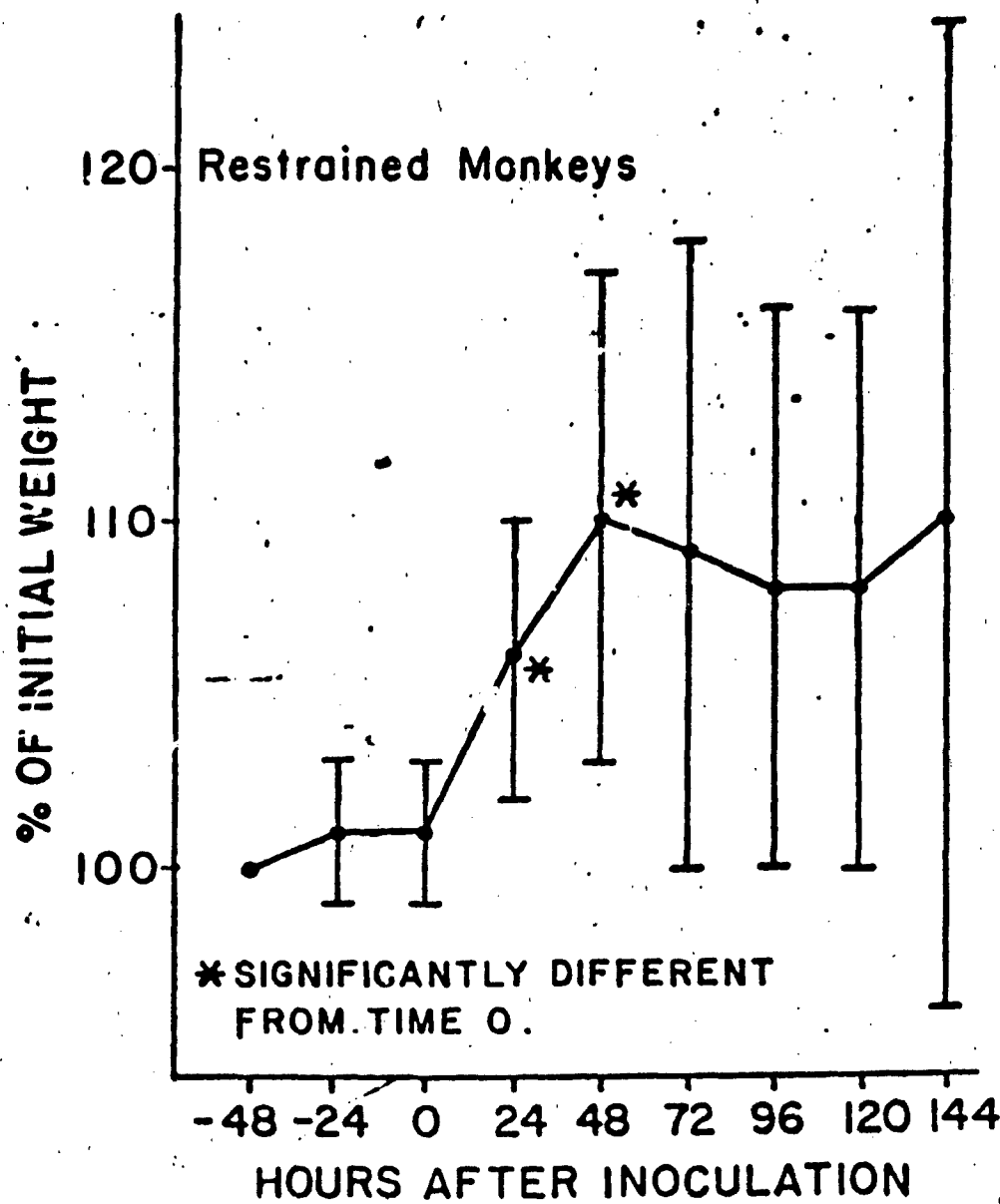
EFFECT OF SEPSIS ON BODY WEIGHT
(% INITIAL WEIGHT)

HOURS AFTER INOCULATION

Figure 1

Fig. 2

Restrained Monkeys

% OF INITIAL WEIGHT

120
110
100

* SIGNIFICANTLY DIFFERENT
FROM TIME 0.
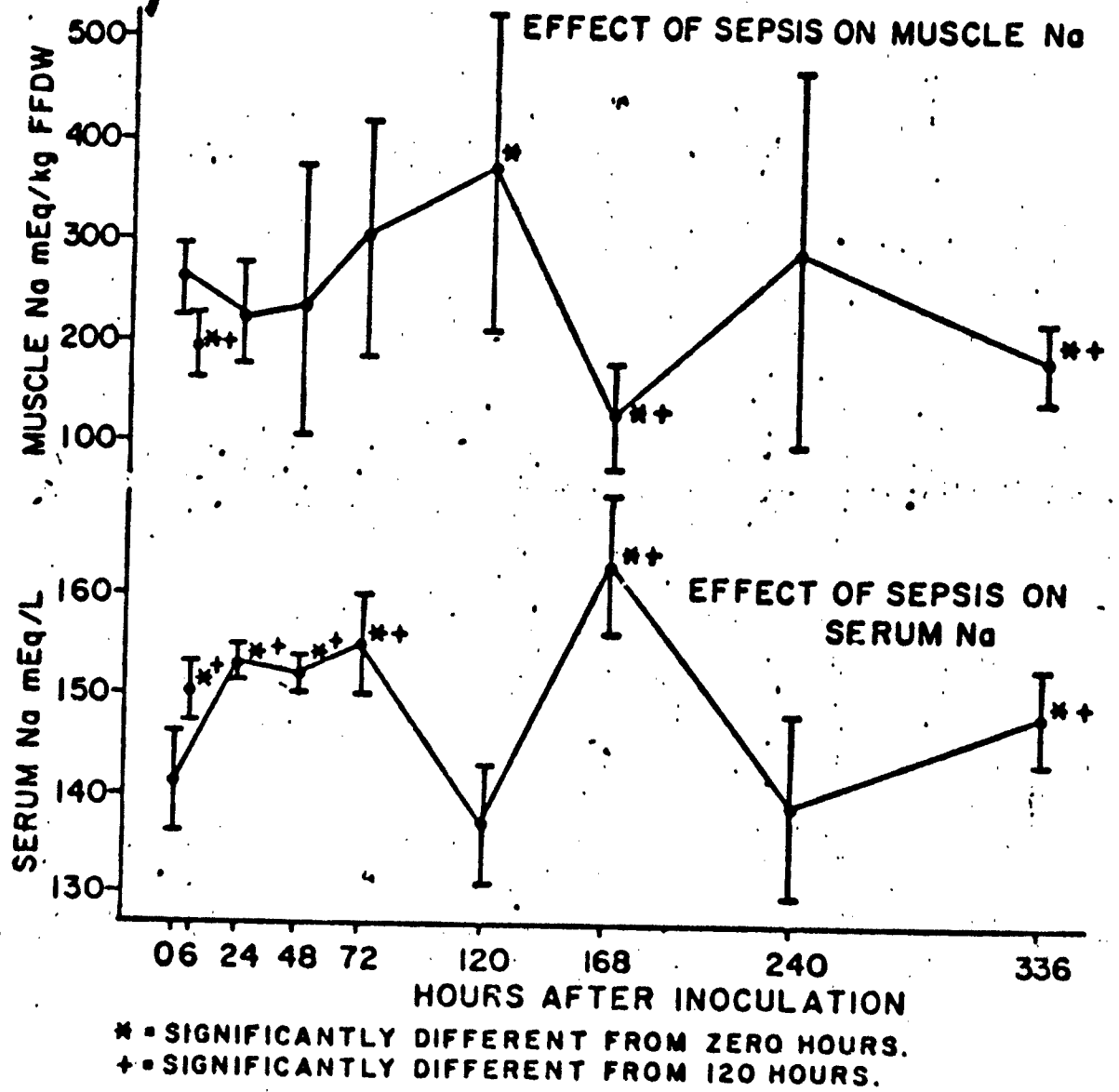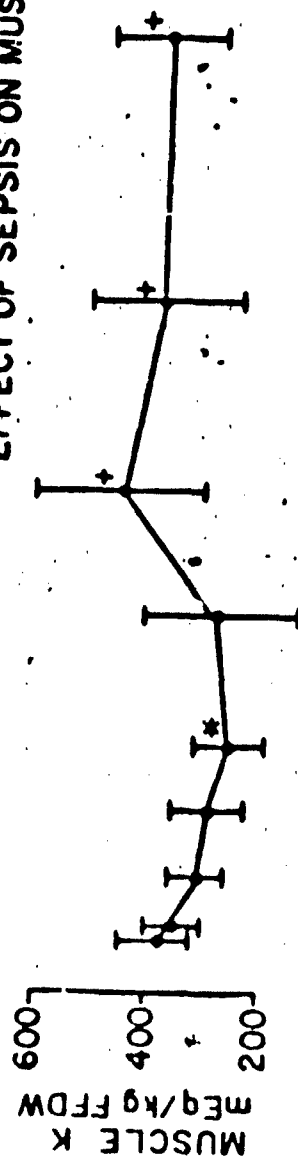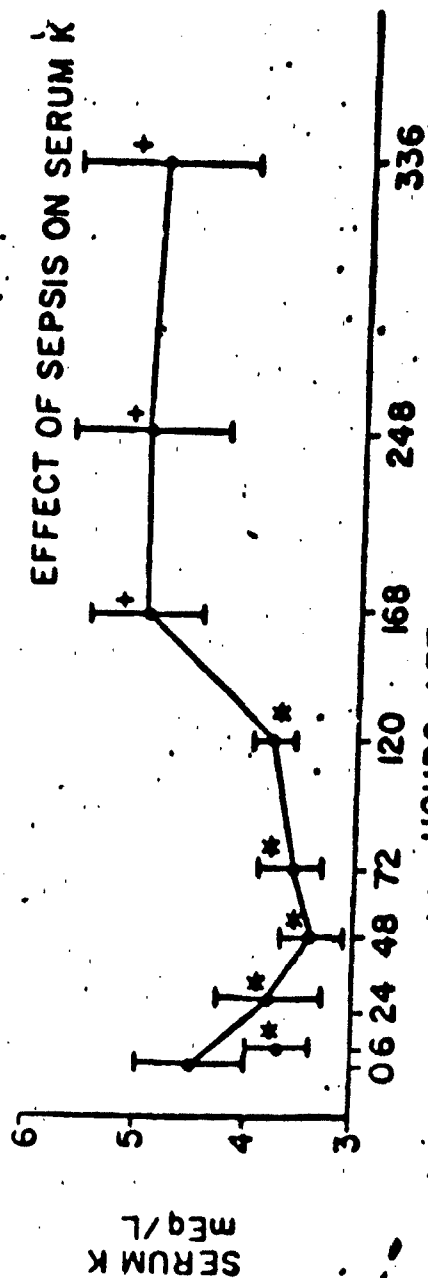
-48 -24 0 24 48 72 96 120 144

HOURS AFTER INOCULATION

EFFECT OF SEPSIS ON MUSCLE Na

EFFECT OF SEPSIS ON SERUM Na

HOURS AFTER INOCULATION

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.
+ = SIGNIFICANTLY DIFFERENT FROM 120 HOURS.

Figure # 3

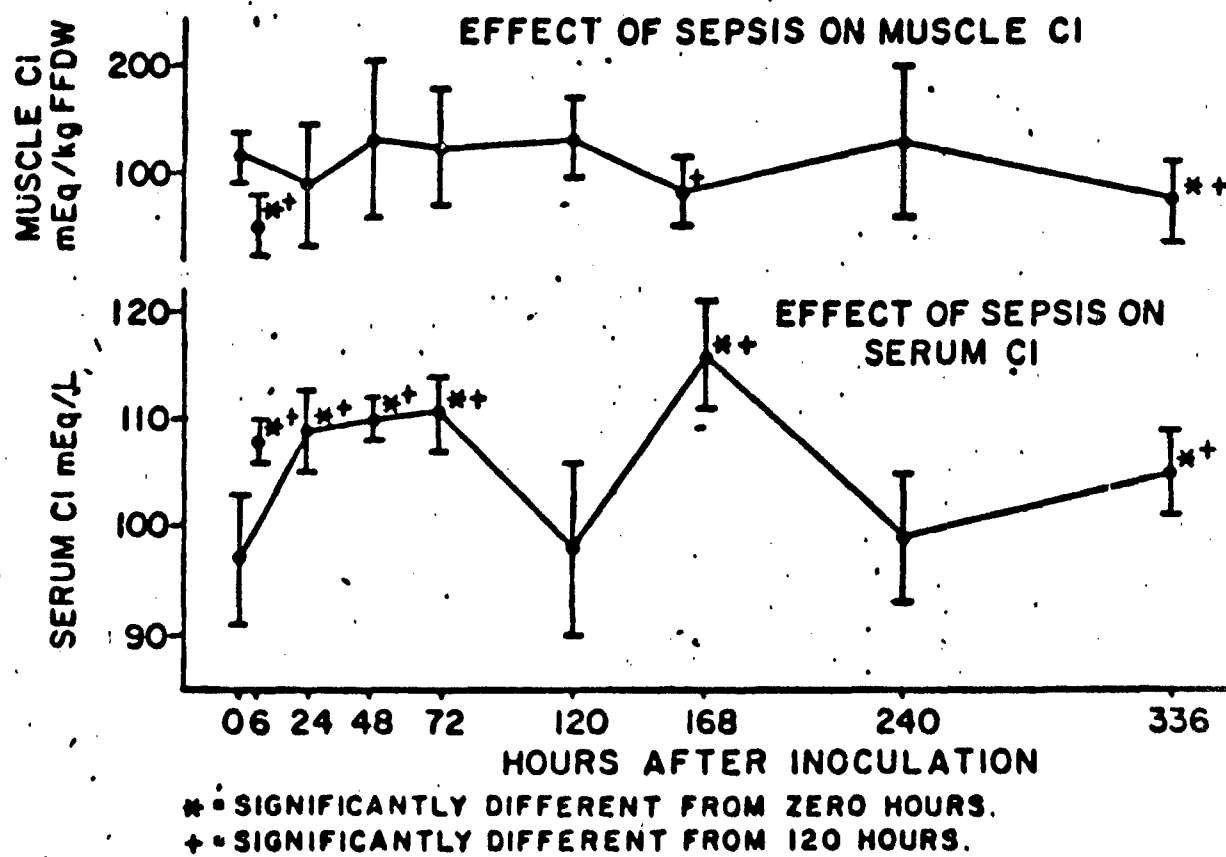EFFECT OF SEPSIS ON MUSCLE K

EFFECT OF SEPSIS ON SERUM K

MUSCLE K
mEq/kg FFDW

SERUM K
mEq/L

HOURS AFTER INOCULATION

0 6 24 48 72 120 168 248 336

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.
+ = SIGNIFICANTLY DIFFERENT FROM 120 HOURS.

Figure 4

EFFECT OF SEPSIS ON MUSCLE Cl

EFFECT OF SEPSIS ON SERUM Cl

MUSCLE Cl mEq/kg FFDW

SERUM Cl mEq/L

HOURS AFTER INOCULATION

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.
+ = SIGNIFICANTLY DIFFERENT FROM 120 HOURS.

Figure # 5

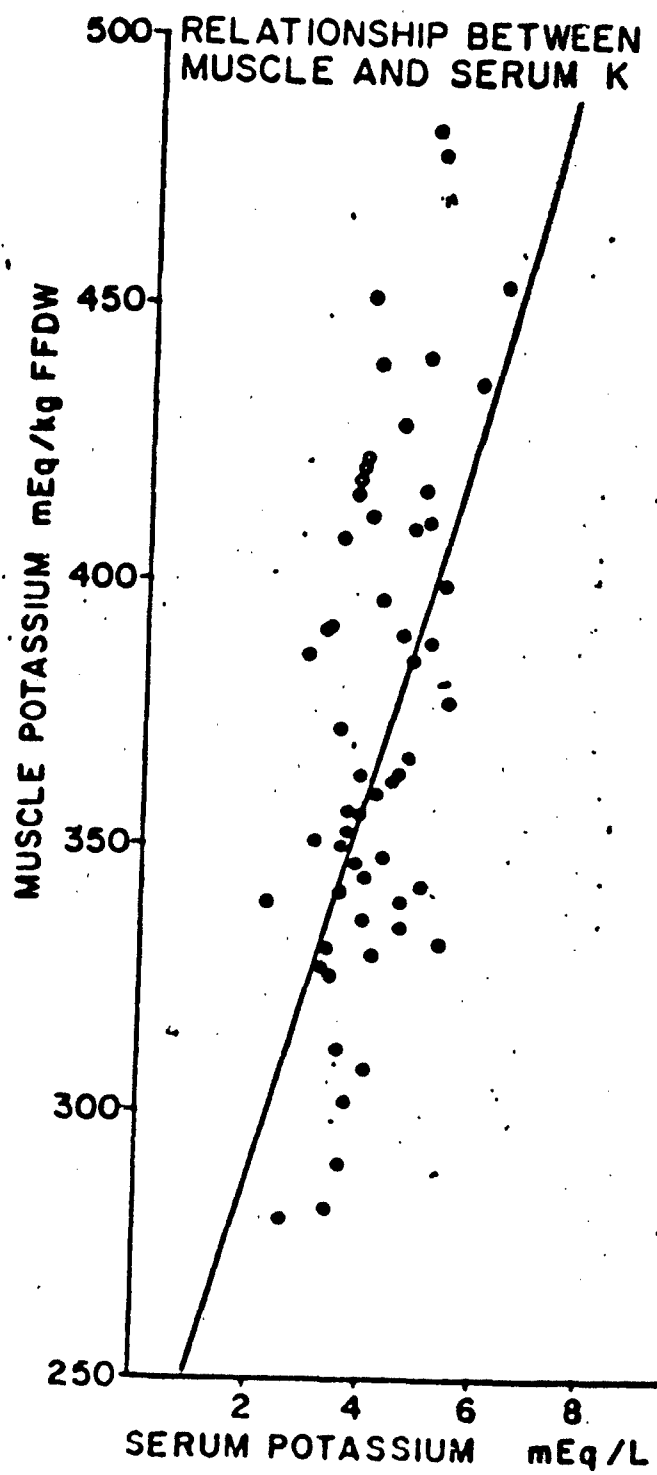RELATIONSHIP BETWEEN MUSCLE AND SERUM K

MUSCLE POTASSIUM mEq/kg FFDW

SERUM POTASSIUM mEq/L

Figure 6

EFFECT OF SEPSIS ON MUSCLE H₂O

EFFECT OF SEPSIS ON MUSCLE MFN CONTENT

EFFECT OF SEPSIS ON MUSCLE COLLAGEN NITROGEN CONTENT

EFFECT OF SEPSIS ON MUSCLE LIPIDS
gm/kg DS

HOURS AFTER INOCULATION

●·SIGNIFICANTLY DIFFERENT FROM ZERO HOURS
◆·SIGNIFICANTLY DIFFERENT FROM 120 HOURS.

Figure 7

Restrained Monkeys

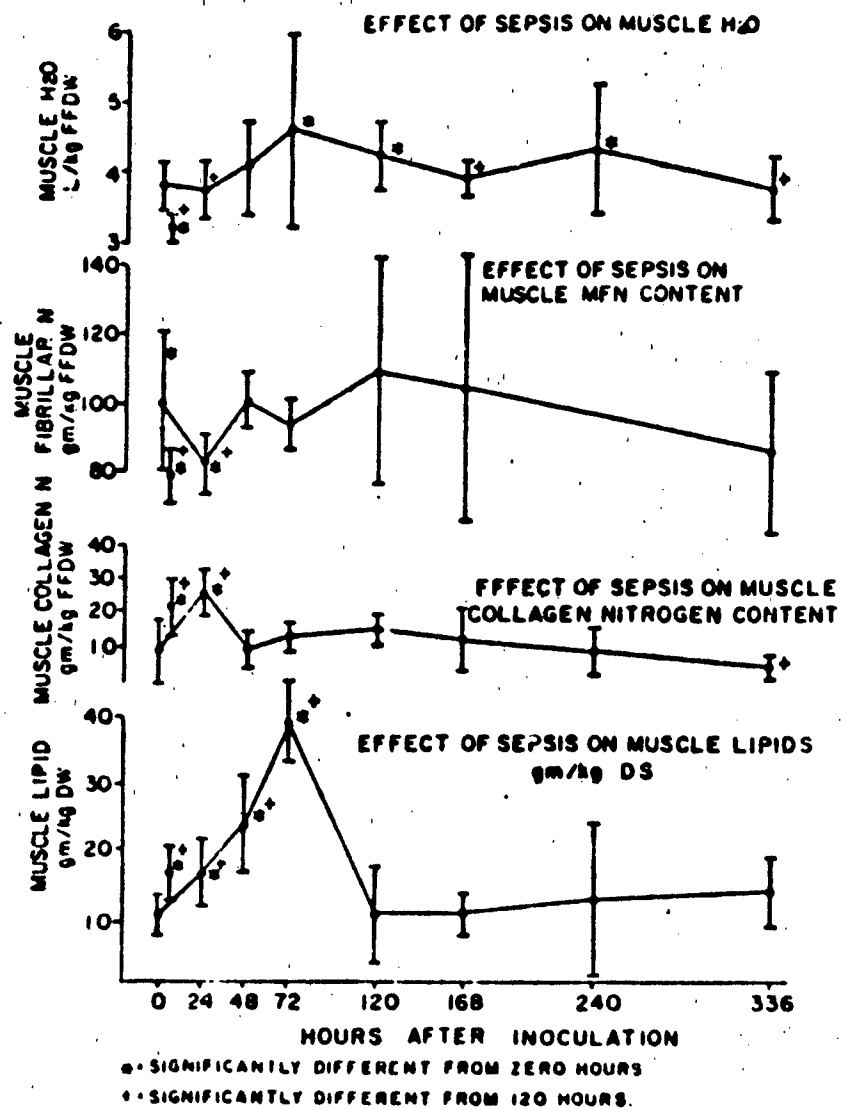URINE Na mEq/24 hrs.

URINE K mEq/24 hrs.

URINE VOLUME ml/24 hrs.

HOURS AFTER INOCULATION

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.

Figure 8

Restrained Monkeys

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.

figure 9

RELATION BETWEEN FIBER WATER AND ELECTROLYTES

Figure # 10

Restrained Monkeys

MUSCLE H₂O L/Kg FFDW.

SERUM mOsm/L

HOURS AFTER INOCULATION

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.

Figure 11

Restrained Monkeys

MUSCLE Na
mEq/Kg FFDW

SERUM Na
mEq/L

HOURS AFTER INOCULATION

* = SIGNIFICANTLY DIFFERENT FROM ZERO HOURS.

Figure 13

Restrained Monkeys

MUSCLE Cl mEq/Kg FFDW

SERUM Cl mEq/L

HOURS AFTER INOCULATION
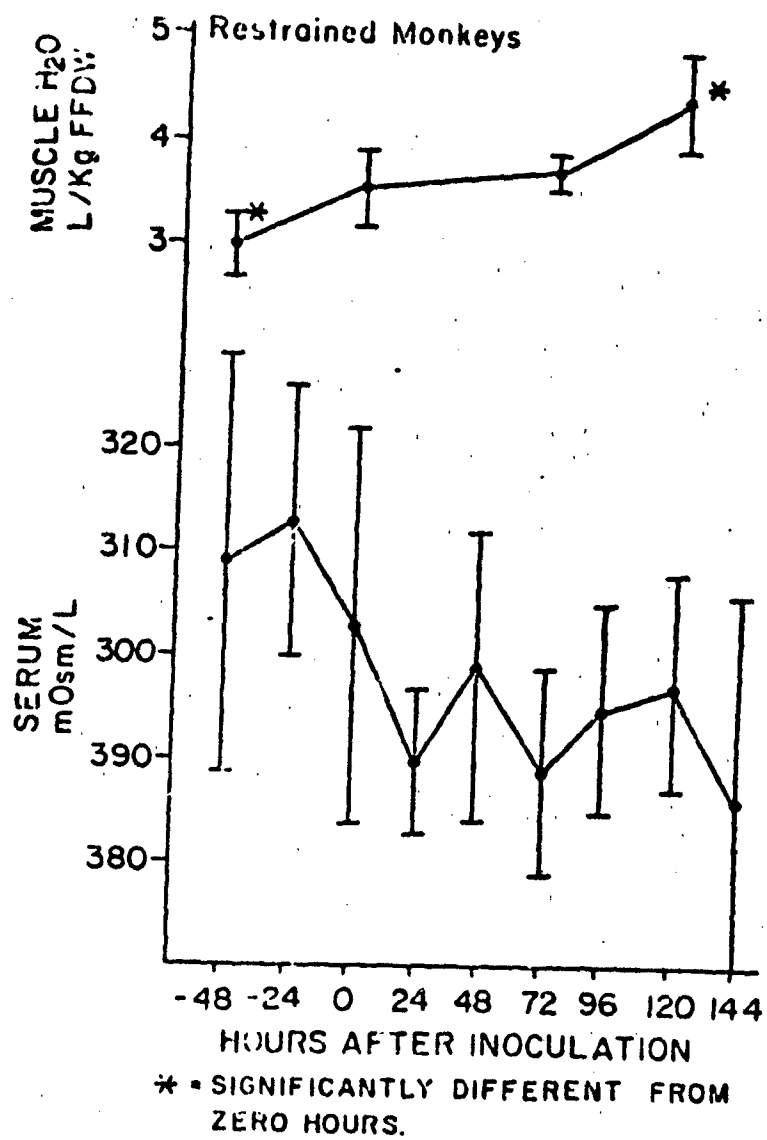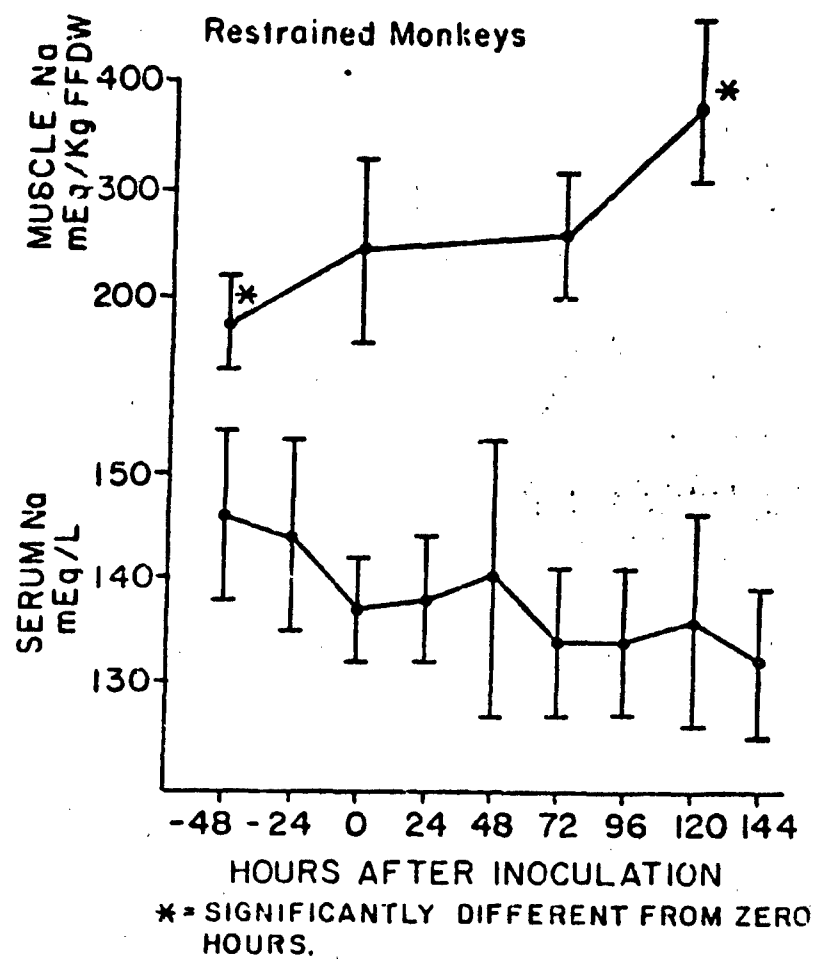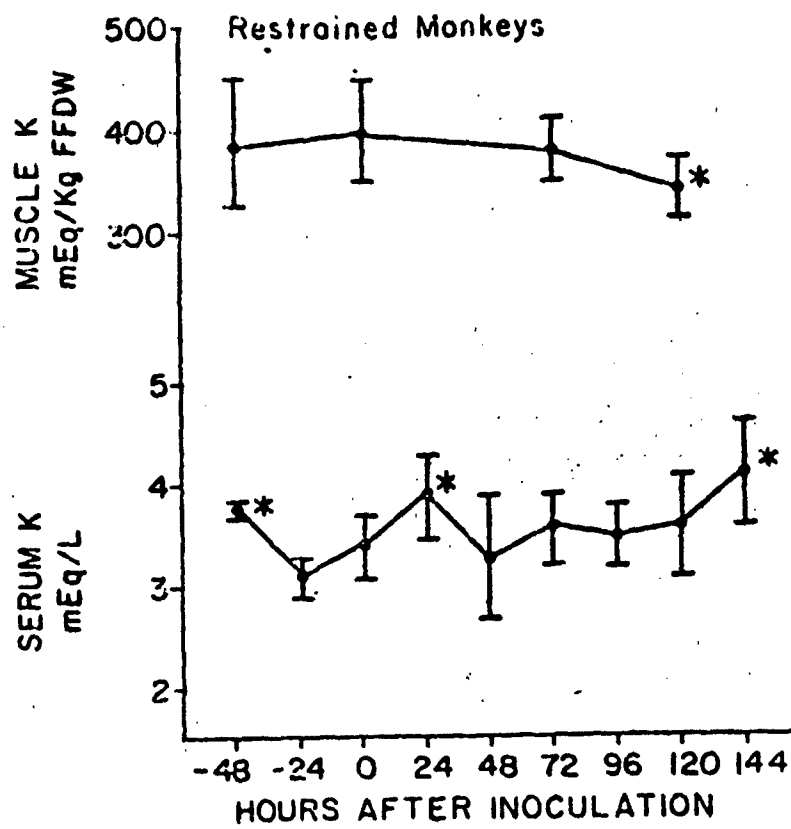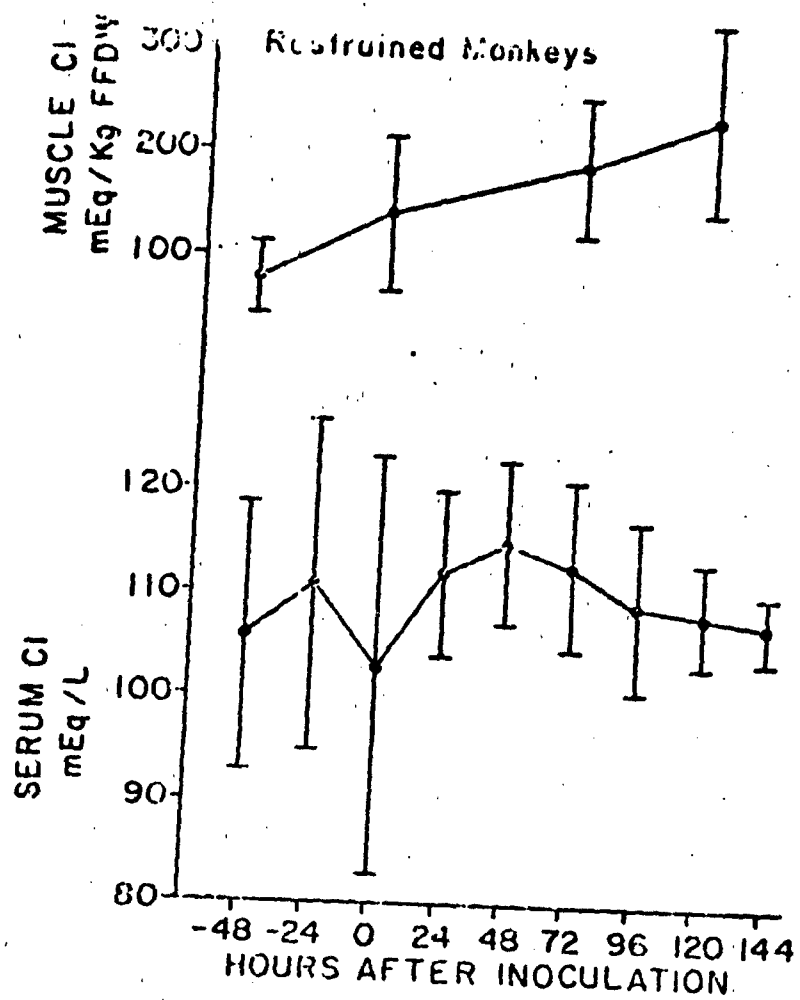
Figure 14

REFERENCES

1. Bilbrey, G. L. and Beisel, W. R. Depression of free water clearance during pneumococcal bacteremia. Annals of Surgery, Vol. 177, No. 1:112, Jan. 1973.

2. Beisel, W. R., Sawyer, W. D., Ryll, E. D. and Crozier, D. Metabolic effects of intracellular infections in man. Annals Int. Med. , Vol. 67, No. 4:744, October 1967.

3. Beisel, W. R., Bruton, J., Anderson, K. D. and Sawyer, W. D. Adrenocortical responses during tularemia in human subjects. J. Clin. Endocr. Metab. Vol. 27:61, January 1967.

4. Beisel, W. R. and Rapoport, M. I. Inter-relations between adrenocorticol functions and infectious illness. N. Eng. J. of Med. Vol. 280, No. 10:541, Mar. 6, 1969.

5. Beisel, W. R. and Rapoport, M. I. Inter-relations between adrenocorticol functions and infectious illness (concluded). N. Eng. J. of Med. Vol. 280, No. 11, Mar. 13, 1969.

6. Nichols, B. L., Bilbrey, G. L., Hazlewood, C. F., Kimzey, S. L., Liu, C. T., Viteri, F., Alvarado, J. and Beisel, W. R. Sequential changes in body composition during infection, electron probe study IV. Am. J. Clin. Nutr. 30:1439, 1977.

7. Nichols, B. L., Spence, D. W., Hazlewood, C. F., Librik, L., Sachen, J. B. and Clayton, G. W. Syndrome characterized by loss of muscle strength experienced by athletes during intensive training programs. Metabolism 21:187, 1972.

8. Nichols, B. L., Soriano, H. A., Sachen, J. B., Burns, L., Hazlewood, C. F. and Kimzey, S. L. Electron probe localization of electrolytes in immature muscle. J. Hopkins Med. J. 135:322, 1974.

9. Fiser, R. H., Denniston, J. C. and Beisel, W. R. Infection with Diplococcus pneumoniae and Salmonella typhimurium in monkeys: changes in plasma lipids and lipoproteins. J. of Inf. Dis. Vol. 125, No. 1:54, January 1972.

10. Kaufmann, R. L., Matson, C. F., Rowberg, A. H. and Beisel, W. R. Defective

lipid disposal mechanisms during bacterial infection in rhesus monkeys. Metabolism
Vol. 25, No. 6:615, June 1976.

11. Wannemacher,Jr., R. W., Powanda, M. C. and Dinterman, R. E. Amino acid flux and
protein synthesis after exposure of rats to either <u>Diplococcus pneumonia</u> or <u>Sal-
monella typhimurium</u>. Infect. and Immun. Vol. 10, No. 1:60, July 1964.

12. Powanda, M. C., Dinterman, R. E., Wannemacher, Jr., R. W. and Herbrandson, G. D.
Distribution and metabolism of phenylalanine and tyrosine during tularemia in
in the rat. Biochem. J. Vol. 144:173, 1974.

TABLE I PHYSIOLOGIC RESPONSE TO S. TYPHIMURIUM SEPTICEMIA

GROUP II SUBJECTS, N = 5

STUDY	0 HOURS	72 HOURS	120 HOURS
BODY COMPARTMENTS			
Plasma Volume % Body Weight	4.0 ± 0.6	4.3 ± 1.1	4.6 ± 0.9
Extracellular Water % Body Weight	26.2 ± 3.6	23.2 ± 3.3	25.7 ± 3.9
CARDIAC FUNCTION			
Cardiac Output ml/min/kg	149 ± 46	140 ± 39	137 ± 46
Pulse beat/min	166 ± 47	176 ± 28	183 ± 15
Mean Arterial Blood Pressure, mm Hg	112 ± 19	97 ± 20	97 ± 20
Peripheral Resistance units	58 ± 13	53 ± 18	55 ± 20
RENAL FUNCTION			
Inulin Clearance ml/min/kg	4.7 ± 1.9	3.3 ± 1.3	3.0 ± 1.6
PAH Clearance ml/min/kg	25 ± 12	19.5 ± 8	23 ± 13
Filtration Fraction C_{In}/C_{PAH}	0.21 ± 0.12	0.18 ± 0.07	0.14 ± 0.02

TABLE II SUMMARY OF GROUP III EXPERIMENTS

DETERMINATION	0 HOURS	TIME AFTER INOCULATION ($M \pm SD$) 120 HOURS	P VALUE
Body Weight, Kg.	3.7 ± 0.5	3.3 ± 0.4*	0.05
Positive Blood Cultures	0/8	8/8	0.01
Hematocrit, Vol. %	39.3 ± 1.8	33.1 ± 4.6	0.01
Serum Proteins, gm/DL	6.9 ± 0.3	6.9 ± 0.4	NS
Blood Urea N, mg/DL	18 ± 3	21 ± 8	NS
Serum Na, mEq/L	150 ± 2	143 ± 5	0.01
Serum K, mEq/L	4.1 ± 0.5	3.2 ± 0.6	0.01
Serum Cl, mEq/L	112 ± 4	105 ± 4	0.01
Serum Osmolality, mOsm/kg	304 ± 4	299 ± 13	NS
Blood pH	7.35 ± 0.02	7.37 ± 0.02	NS
Serum Ca, mg/DL	9.6 ± 0.5	9.0 ± 0.4	0.01
Serum Mg, mg/DL	1.81 ± 0.16	1.82 ± 0.29	NS
Serum P, mg/DL	4.6 ± 0.7	3.0 ± 0.3	0.01
Muscle Na, mEq/Kg FFDW	141 ± 39	170 ± 92	NS
Muscle K, mEq/Kg FFDW	396 ± 33	351 ± 57	0.05
Muscle H_2O, L/Kg FFDW	3.1 ± 0.1	3.4 ± 0.7	NS
Muscle Na mEq/Kg-FFHW	34 ± 9	37 ± 12	NS
Muscle K, mEq/Kg FFHW	95 ± 6	86 ± 13	NS
Fiber Na, mEq/Kg WW	20 ± 7	20 ± 7	NS
Fiber K, mEq/Kg WW	104 ± 11	85 ± 9	0.01
Fiber Cl, mEq/Kg WW	9 ± 6	11 ± 5	NS

TABLE III   EVALUATION OF EFFECTS OF EXPERIMENTAL PROCEDURES ON ELECTROLYTE COMPOSITION

DETERMINATIONS	ABSOLUTE CONTROLS	EXPERIMENTAL CONTROLS	OPERATED CONTROLS
Serum Na, mEq/L	150 ± 3	141 ± 5*	137 ± 5*
Serum K, mEq/L	4.1 ± 0.5	4.6 ± 0.5	3.4 ± 0.3*
Serum Cl, mEq/L	97 ± 6	112 ± 4*	104 ± 20
Serum Osmolality, mOsm/kg	304 ± 4	----	303 ± 19
Muscle H_2O, L/kg FFDW	3.2 ± 0.3	3.8 ± 0.4*	3.5 ± 0.4*
Muscle Na, mEq/kg FFDW	175 ± 44	229 ± 15*	245 ± 85*
Muscle K, mEq/kg FFDW	378 ± 42	378 ± 76	397 ± 52
Muscle Cl, mEq/kg FFDW	52 ± 28	83 ± 45*	140 ± 73*

Absolute Controls: Monkeys never before studied, Group I, time -72 hours and Group III, time zero.

Experimental Controls: Monkeys previously subjected to experimental procedures including blood and muscle sampling, Group I, -48, -24 and zero hours.

Operated Controls: Monkeys subjected to chronic cannulations and restraint in metabolic chairs, Group II, -48, -24 and zero hours.

*Significantly different from Absolute Control mean, p <0.01